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PYROLYSIS GAS CHROMATOGRAPHY OF AMINO ACIDS AND PROTEINS

by
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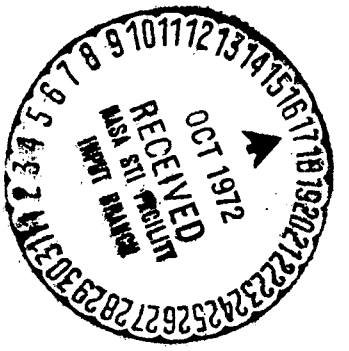

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Aminosan, Tampakushitsu No Netsubunkai Gasukuromatogurafi

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PYROLYSIS GAS CHROMATOGRAPHY OF AMINO ACIDS AND PROTEINS

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Kazuo Kanomata*1 and Yoichiro Mashiko*2

Pyrolysis gas chromatography was performed to estimate quickly and simply the amino acid composition. The column A, which was constructed with three kinds of columns (hexanedion, tetraethyleneglycol-dimethylether, dioctylphthalate), and column B (silicone D.C. 550) were used as columns. Considering that the ratios of the peak areas of pyrolysis gas chromatography patterns of eighteen kinds of amino acids differ according to their types, it was concluded that they should be compared with the pyrolysis patterns of amino acids. By using milk-casein and egg-albumin as protein samples, the amino acid composition was estimated from the ratios of the peak areas and the types. To confirm the results, the protein samples were analyzed in the amino acid analyzer and compared. They were found to be nearly identical except for a few amino acids whose patterns were less distinctive.

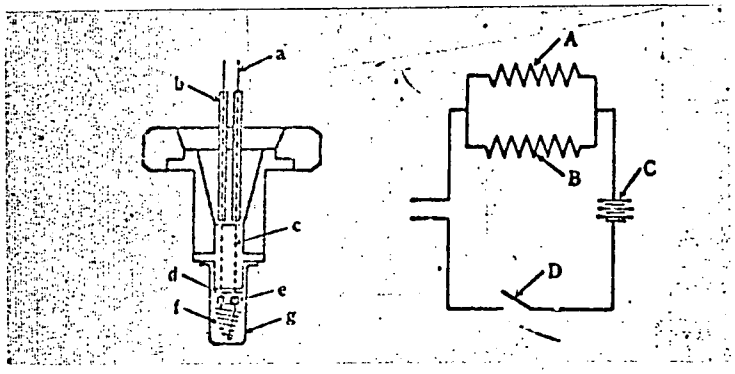
1. Introduction

Pyrolysis gas chromatography is mainly used for the analysis of the synthetic macromolecules. There are various reports concerning the methods of analysis: the method in which the materials previously thermo decomposed in the glass tube are introduced into the gas chromatography apparatus [1-4];

the method in which the thermo decomposition cell is connected with the sample cell [5-7]; or the method of direct analysis by filament (8-14).

Figure 1. I Wiring connected to a platinum wire basket.

II Pyrolysis apparatus in which the platinum wire basket is set at the sample inlet.



I II

a) copper wire b) porcelain insulator tube c) silicon gum d) mica-plate e) porcelain insulator tube f) platinum wire g) outer tube (stainless), A,B: nichrome wire (1.1 ohm), C: battery (6V, 60 amp-hr), D: electric key-switch

In this report, the method of direct analysis [14] by Mashiko, Echizen, and Nakao was employed to obtain the pyrolysis gas chromatography patterns of amino acids and proteins, and by comparing both of them the experiment was conducted to estimate the amino acid composition of proteins.

2. Experiment

2.1 Construction of Pyrolysis Apparatus

The basket (figure 1) made of coiled 0.2 mm x 5 cm platinum wire (1.5 ohm) was set at the sample inlet of the gas chromatography apparatus (Shimadzu, type GC-2A), in which a nichrome wire (1.1 ohm) was put in series with the platinum wire, connected to the battery (6V,60 amp-hr). The red heat of the basket was activated by pushing the switch D.

2.2 Preparatory Experiment

2.2.1 Selection of Plugging Materials

To determine the type of plugging material, about 10 mg of glutamic acid (paper chromato reagent, Ajinomoto Co. L.T.D.) was used as a carrier gas (flow rate 65 ml/min; temperature 21°C) and the switch was pushed after the air peak appeared, decomposing the acid for 20 seconds.

The use of 19 types of the plugging materials in order to compare the patterns showed that hexanedione, tetraethylenglycoldimethylether, dioctylphthalate were satisfactory both in the number of the peaks and in the condition of separation.

2.2.2 Determination of Flow Rate

The column which was obtained from 2.2.1 was used and a pattern was obtained by chang-

ing the flow rate of the carrier gas from 20 to 40, 60, 100, and 120 ml/min (temperature 20°C; analysis time 20 seconds). The rate 60 ml/min proved satisfactory from the pattern of the peak and the condition of separation. Under these conditions, 1200 was about the largest theoretical step number.

2.2.3 Determination of the Column Temperature

When an experiment was conducted at temperatures of 21°, 30°, 40°, 60°C, the separation of the peaks above 30°C was not satisfactory enough and the peak pattern satisfactory enough to measure the area was not obtained; thus, the temperature of the column was determined to be 21°C.

2.2.4 Determination of Pyrolysis Time

When the number of the peaks which were obtained by setting the analysis time in ten steps, from two to sixty seconds, was explored, it proved satisfactory between ten and twenty seconds, thus, the analysis time was determined to be 15 seconds (2.2.1-3)

From the results of preparatory experiment the conditions of the experiment were determined as follows:

1) The column constructed of hexanedione (1/4 in x 2m), tetraethyleneglycoldimethylether (1/4 in x 2m), and dioctylphthalate (4mm x 3m) was used;

it was connected in the above order from the side of the sample injection.

2) Helium was used as the carrier gas (flow rate 65 ml/min, temperature 21°C, and pyrolysis time 15 seconds).

2.3 Actual Experiment

Of the eighteen types of amino acids (reagents for paper Chromato by Ajinomoto Co. L.T.D.), about 10 mg from each amino acid was used, and pyrolysis gas chromatography was performed.

Considering that phenylalanine, tyrosine, and triptophan have a benzene nucleus, that will produce benzene derivatives, pyrolysis gas chromatography was performed again in the column of Silicone D.C. 550 (4mm x 3m) which was prepared separately; helium flow rate was 65 ml/min, temperature--50°. On the basis of the obtained pyrolysis gas chromatography patterns of amino acids, milk-casein (by Meruku) and egg-albumin were used as protein samples to estimate the amino acid composition. 20 mg was taken from each protein sample and thermo decomposed under the same conditions as the amino acids, thus the pattern from both columns of hexanedione, etc., and silicone was obtained.

3. Results

The patterns of each amino acid is shown in figure 2; each of them has all or some of the six peak types in regard to the maintenance time; 9.0 minutes (peak A), 12.4 minutes (peak B), 15.5 minutes (peak C), 19.1 minutes (peak D), 23.6 minutes (peak E), 29.5 minutes (peak F); peak C was the largest of any amino acids.

In regards to the patterns of each amino acid, each peak area was measured; the sum of each peak was considered 1; and the percentage of each peak was calculated. The pattern of phenylalanine which was analyzed in the Silicone D.C. 550 column showed the peak of benzene at the maintenance time of 14.5 minutes, and that of toluen at 33.5 minutes. In tyrosine, the pattern showed the benzene peak (14.5) and phenol peak (53.5 minutes). In the same way, triptophan showed these peaks of benzene, toluen, and phenol.

The patterns which thermo decomposed milk-casein and egg-albumin are shown in figures 3 and 4, and the area ratios of the peaks made by the columns of hexanedione and others are shown in the figure 2. By comparing figure 1 and 2 the amino acid composition of the protein samples was estimated as follows:

In regards to milk-casein:

1) from the area ratios between A and C, as the number of A is comparatively large, alanine, valine, lysine, histidine, and proline, which correspond to this condition in chart 1, were identified.

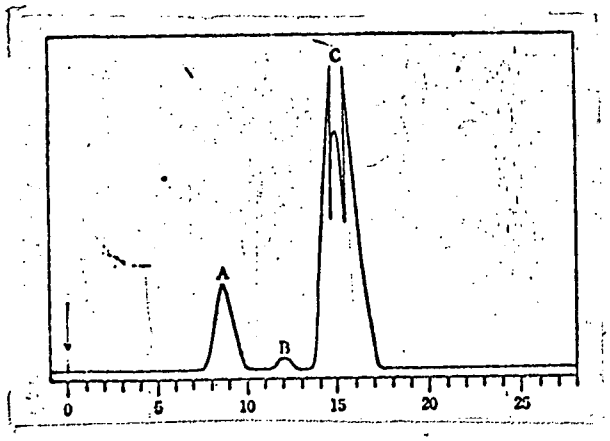
2) when B and C were compared, B was

relatively larger than C; A was also relatively large. Thus, from Chart 1 alanine, lysine, arginine histidine, and proline were identified.

3. In peak D, a comparatively large number was obtained, so valine and glutamic acid which corresponded to the number were identified.

Chart 1 The Peak Area Ratios of Each Amino Acid (%)

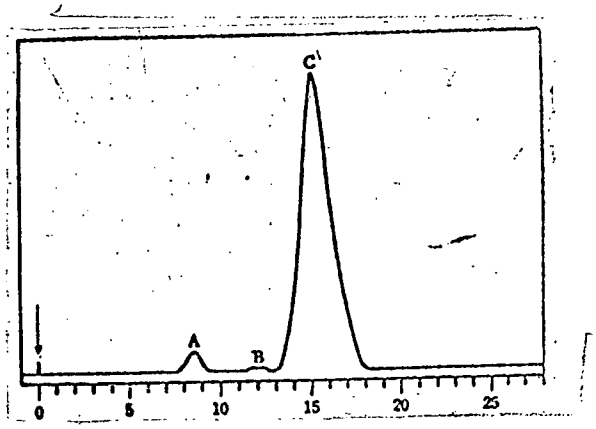
The Name of Amino Acids	Designation of the Peaks					
	A	B	C	D	E	F
DL-alanine	13.20	1.16	85.60	0	0	0
L-valine	20.70	0.62	73.90	0.37	4.47	0
L-leucine	9.90	0.57	86.40	+	3.40	0
glycine	6.42	0.27	93.40	0	0	0
L-serine	3.13	0.37	96.50	0	0	0
L-threonine	1.18	0.08	98.30	+	0.40	0
L-lysine	16.00	3.11	81.00	+	+	0
L-arginine	9,87	5.58	83.50	+	0.95	0
histidine	15.60	1.25	83.20	0	0	0
L-proline	11.05	8.65	78.80	+	1.40	0
L-oxyproline	7.40	1.59	91.00	+	+	0
aspartic acid	3.96	0.21	95.80	±	0	0
glutamic acid	5.87	0.79	93.00	0.44	0	0
cystine	2.45	1.22	96.00	+	0.16	0.10
L-methionine	6.37	1.47	91.70	±	0.66	+
phenylalanine	3.70	±	96.00	0	0	0
tyrosine	2.08	0	97.80	0	0	0
tryptophan	1.56	0	98.50	0	0	0



Maintenance Time (min)

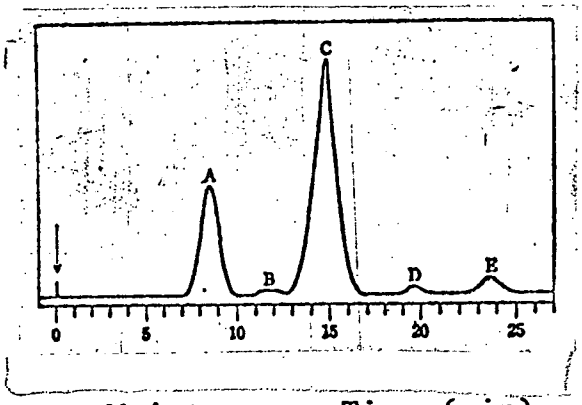
The arrow shows the pyrolysis (same in each figure)

Figure 2.1 DL-alanine



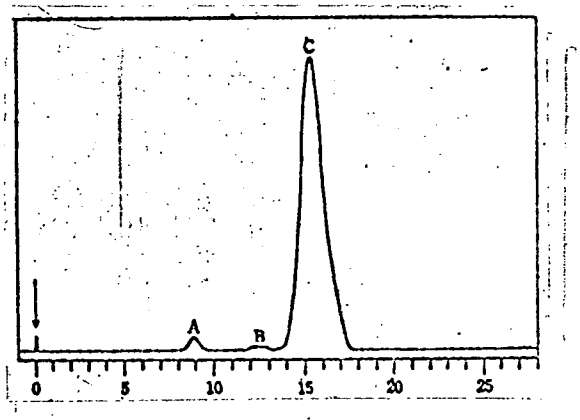
Maintenance Time (min)

Figure 2.4 glycine



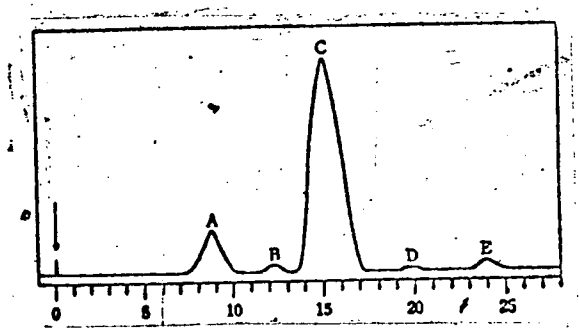
Maintenance Time (min)

Figure 2.2 L-valine



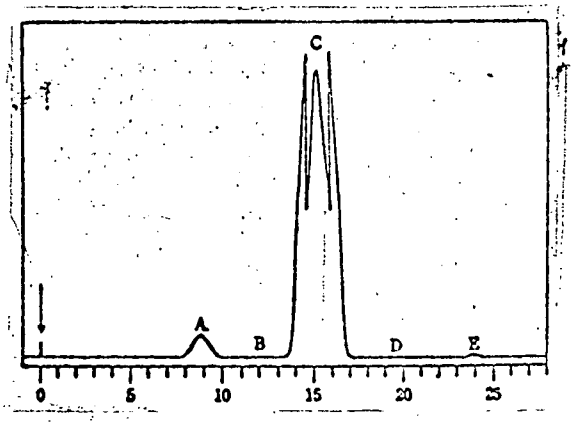
Maintenance Time (min)

Figure 2.5 L-serine



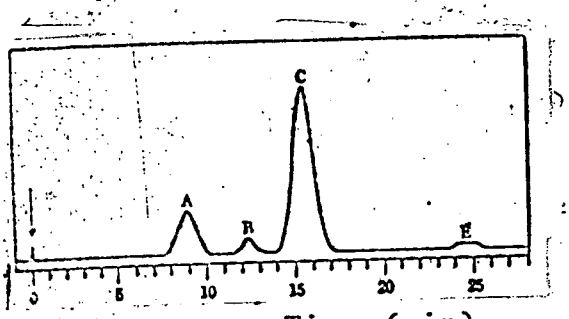
Maintenance Time (min)

Figure 2.3 L-leucine



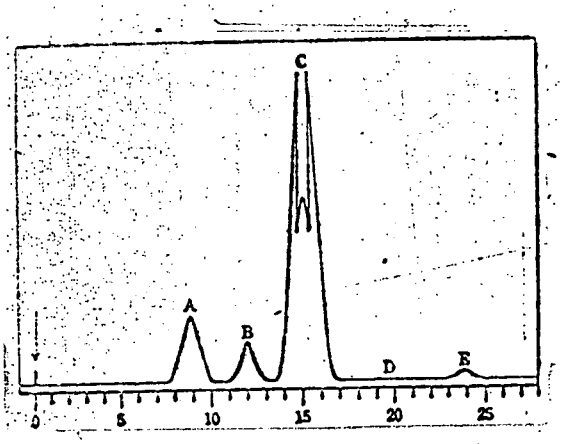
Maintenance Time (min)

Figure 2.6 L-lysine



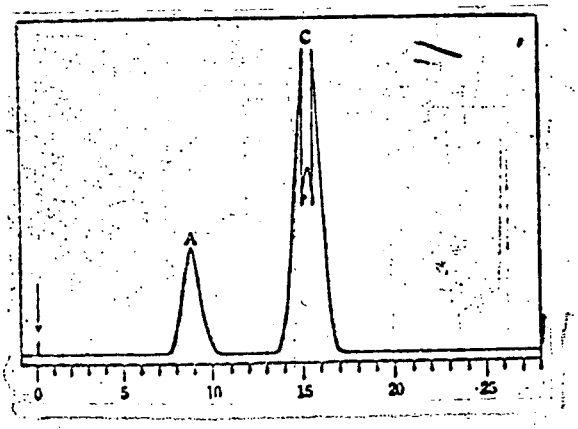
Maintenance Time (min)

Figure 2.7 L-threonine



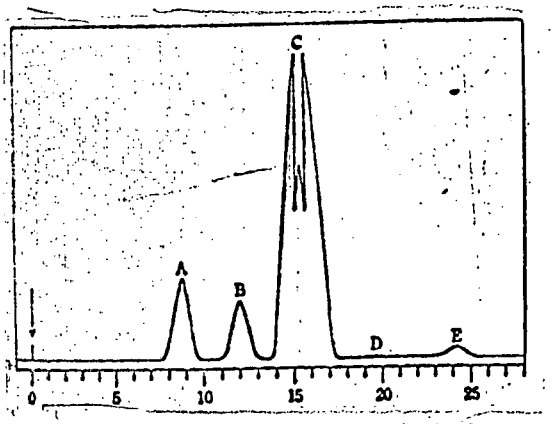
Maintenance Time (min)

Figure 2.8 L-arginine



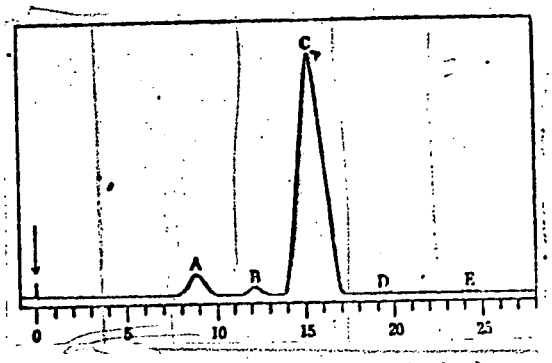
Maintenance Time (min)

Figure 2.9 histidine



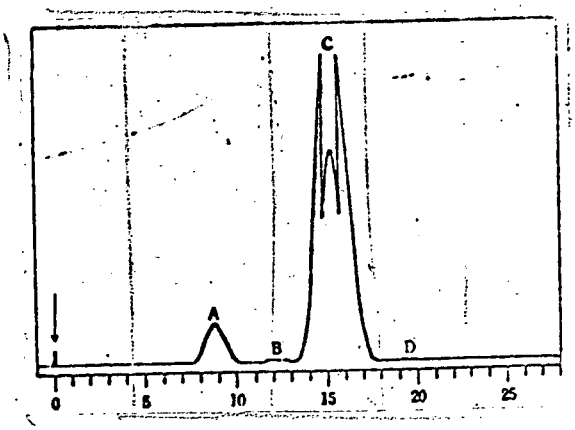
Maintenance Time (min)

Figure 2.10 L-proline



Maintenance Time (min)

Figure 2.11 L-oxyproline



Maintenance Time (min)

Figure 2.12 aspartic acid

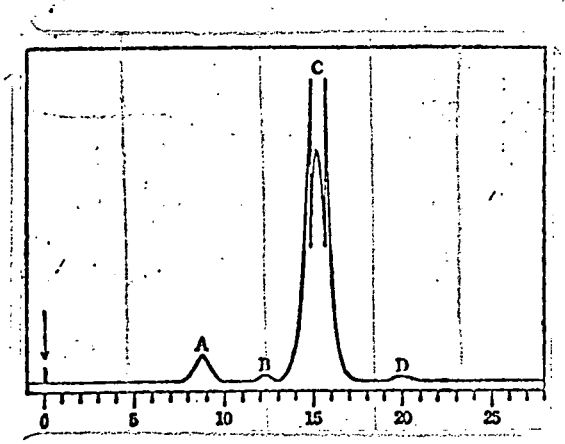


Figure 2.13 glutamic acid

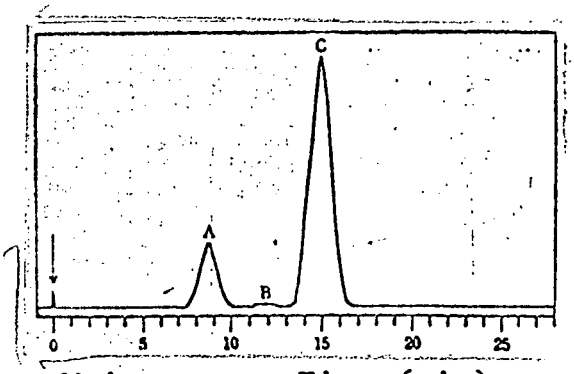


Figure 2.16 Phenylalanine (1)

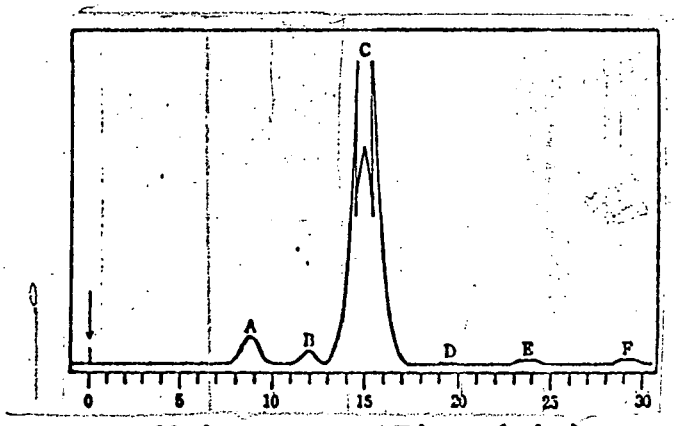
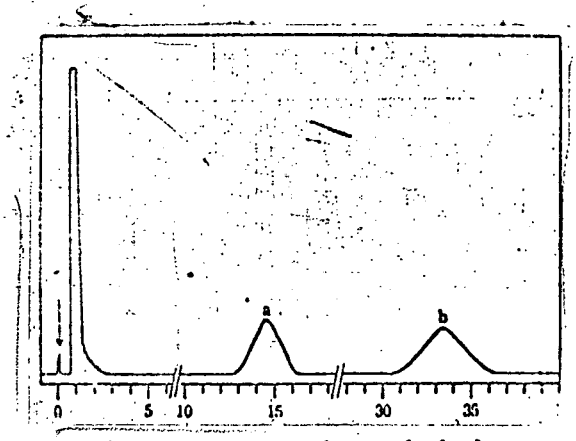


Figure 2.14 cystine



column: silicone D.C. 550
a: benzene, b: toluen
Figure 2.17 phenylalanine (2)

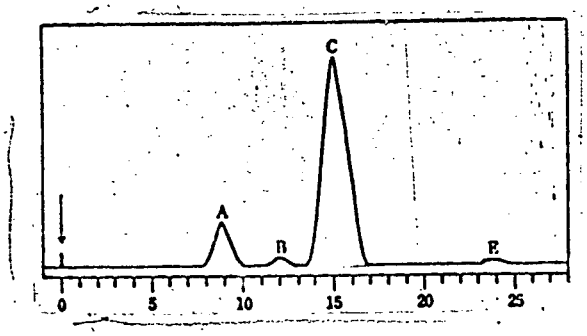


Figure 2.15 methionine

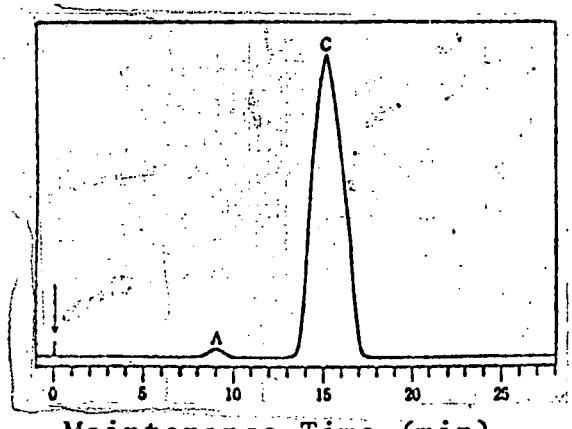
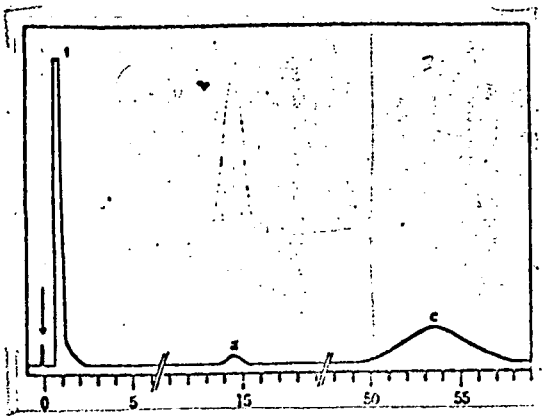
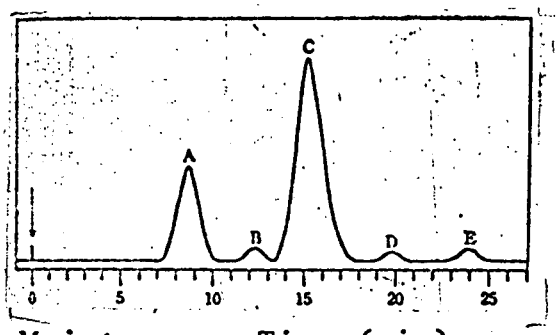


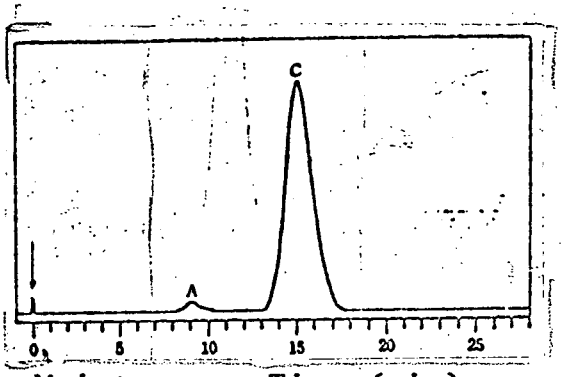
Figure 2.18 tyrosine (1)



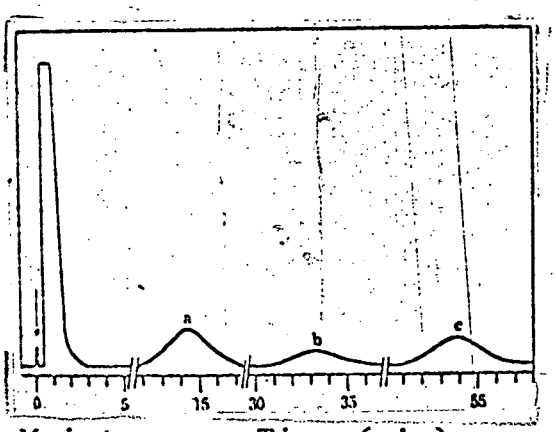
Maintenance Time (min)
column: silicone D.C. 500
a: benzene, c: phenol
Figure 2.19 tyrosine (2)



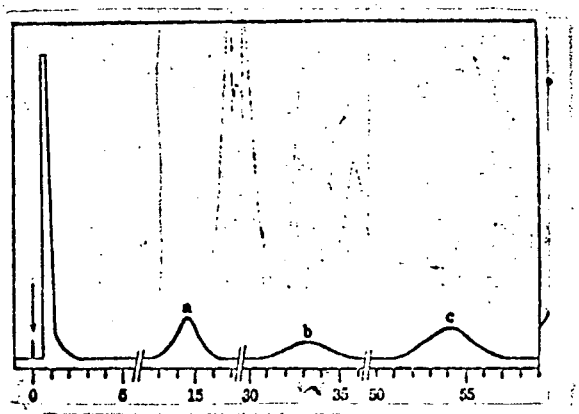
Maintenance Time (min)
Figure 3.1 milk-casein (1)



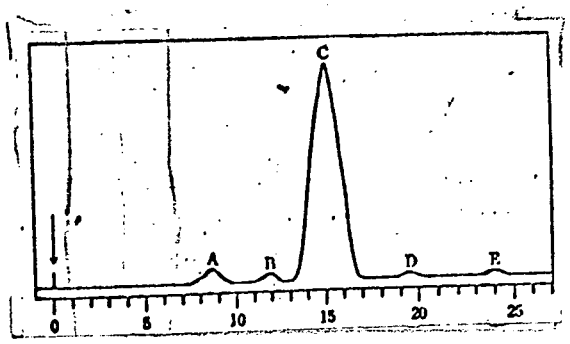
Maintenance Time (min)
tryptophan (1)
Figure 2.20



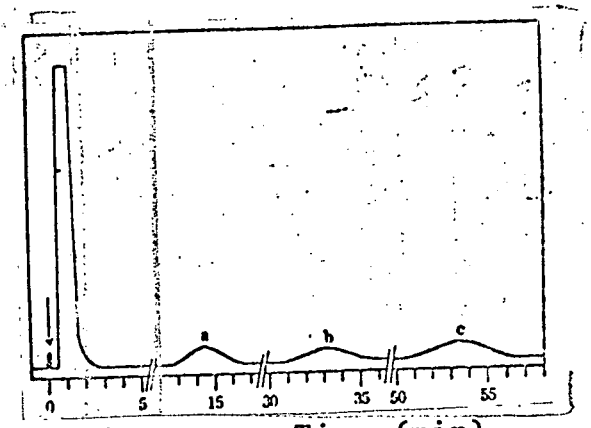
Maintenance Time (min)
column: silicone D.C. 550
a: benzene, b: toluen
c: phenol
Figure 3.2 milk-casein (2)



Maintenance Time (min)
column: silicone D.C. 550
a: benzene, b: toluen
c. phenol
Figure 2.21 tryptophan (2)



Maintenance Time (min)
Figure 4.1 egg-albumin (1)



Maintenance Time (min)
column: silicone D.C. 550
a: benzene, b: toluen
c: phenol
Figure 4.2 egg-albumin (2)

Chart 2 The Peak Area Ratios of the Protein Samples (%)

The Name of the Samples	Designation of the Peaks					
	A	B	C	D	E	F
milk-casein	19.80	4.30	--	--	4.27	0
egg-albumin	3.62	0.97	--	--	0.32	+

4. Because the number of peak E is large and there is no peak F, valine, leucine, threonine, arginine, proline, and methionine (which has such traces in F that its area cannot be measured and has a peak in E) were identified from Chart 1. Next, a pyrolysis was performed under the column of silicone D.C. 550, and phenylalanine, tyrosine, and triptophan were identified from the obtained patterns.

In regard to egg-albumin, 1) glycine, serine, threonine, oxyproline, aspartic acid, glutamic acid, cystine, and methionine were identified from Chart 1, because the number of C was larger when

peak A and C were compared. 2) since peak E was present, valine leucine, threonine, arginine, proline glutamic acid, cystine, and methionine were identified. 4) since it had peak F, cystine and methionine were identified. 5) since it had both peak D and E, valine, leucine, threonine, lysine, arginine, proline, oxyproline, cystine, and methionine were identified. According to the column of silicone D.C. 550, the existence of phenylalanine, tyrosine, and triptophan were estimated. From these results, the amino acid composition in milk-casein was estimated to be alanine, valine, leucine, threonine, lysine, arginine, hestidine, proline, glutamic acid, methionine, phenylalanine, tyrosine, and triptophan. In regard to egg-albumin, it was estimated to be valine, leucine, glycine, serine, threonine, lysine, arginine, proline, oxproline, aspartic acid, glutamic acid, cystine, methionine, phenylalanine, tyrosine, and triptophan. Next, the samples were analyzed in the amino acid analyzer (Beckman, type 120 B), and the results were compared in chart 3 to the results estimated by the pyrolysis method. Ammonia and isoleucine were not identified because they were not tested by the pyrolysis method, but it was learned that almost all amino acid compositions can be estimated relatively simply and quickly from the patterns of pyrolysis

gas chromatography by exploring and judging synthetically the peak A, the ratio of C, the identification of the unique peaks, D, E, F and the peak which was estimated by the column of silicone.

4. Summary

4.1 Columns hexanedione (1/4 in x 2m) tetraethyleneylycol dimethylether (1/4 in x 2m) dioctylphthalate (4mm x3m) were connected, and when pyrolysis gas chromatography of the eighteen types of amino acids was performed under the conditions where a carrier gas was helium (65 ml/min; temperature 21°C; pyrolysis time - 15 sec.), a pattern was obtained which had peaks at all or some of the maintenance times: 9.0, 12.4, 15.5, 19.1, 23.6, 29.5 minutes.

4.2 When the column was silicone D.C. 550 (4 mm x 3m; temperature - 50°C; carrier gas, helium 65 ml/min), phenylalanine, tyrosine, and triptophan produced the peaks of benzene, toluene and phenol.

4.3 An experiment was conducted using milk-casein and egg-albumin as samples to estimate the amino acid composition of proteins from the patterns of pyrolysis gas chromatography. It was difficult to estimate the existence of histidine, glycine, and serine whose number of peaks is small, and whose area ratios are similar, showing no characteristic peaks. However, other amino acids were closely estimable.

Chart 3 The comparison of the amino acid components of the samples with estimates by pyrolysis

The Name of Amino Acids	milk-casein		egg-albumin	
	analysis value (%)	estimate results	analysis value (%)	estimate results
lysine	7.8	++	10.1	+
histidine	2.9	+	4.1	inestimable
ammonia	5.1		5.4	
arginine	4.4	++	10.9	++
oxyproline	---	--	0.1	++
aspartic acid	4.3	inestimable	6.0	+
threonine	4.8	+	3.5	++
serine	5.0	inestimable	6.9	+
glutamic acid	21.8	++	10.2	++
proline	10.2	++	8.5	++
glycine	1.5	inestimable	3.8	±
alanine	3.0	+	3.0	—
cystine	0.1	—	0.4	++
valine	7.0	++	5.2	++
methionine	3.0		2.9	++
isoleucine	4.9	+	4.9	
leueine	9.2	+	6.0	+
tyrosine	5.7	+	3.3	+
phenylalanine	5.3	+	4.2	+
triptophan	1.3	+	1.2	+

We would like to express our gratitude to the technical officials of Tokyo Research Industry Laboratory: Mr. Hiroshi Tomita, Mr. Ichiro Takeda who were of great help to us during the experiments, and also Mr. Akio Harada, an engineer, of Aichi Prefectural Food Research Laboratory, who conducted the analysis of the amino acids.

(1963, April, the 16th annual lecture of Japan Chemical Society)

References

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